IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	'COMPOUNDS'
HUGH CAIRNS et al	:	Group Art Unit 122
Serial No 946,492	:	David Wheeler, Examiner
Filed 28th September 1978	:	

DECLARATION

I RAYMOND WILLIAM KEOGH, declare and say as follows:

I am a subject of the Queen of Great Britain and reside at 234, Broome Lane, East Goscote, Leicestershire, England.

I have obtained the degree of Bachelor of Science (Medical Biochemistry) (1966) and Doctor of Philosophy (Experimental Pathology) (1970) from the University of Birmingham, England. I am also a Member of the British Society for Immunology.

Since 1970 I have worked in the Research and Development Laboratories of the Pharmaceutical Division of Fisons Limited and currently hold the post of Head of Life Sciences in those Laboratories.

The following test has been carried out under my supervision. Charles River France/Fisons bred rats (male or female) having a body weight of from 100 to 150g were infected subcutaneously at weekly intervals with N. brasiliensis larvae in doses increasing from about 2000 larvae per animal to 12000 larvae per animal in order to establish the infection. After 8 weeks the rats were bled by heart puncture and 15-20 mls of blood collected from each animal. The blood samples were then centrifuged at 3500 rpm for 30 minutes in

order to remove the blood cells from the blood plasma. The serum was collected and used to provide a serum containing N. brasiliensis antibody. A pilot sensitivity test was carried out to determine the least quantity of serum required to give a skin weal in control animals in the test decribed below of 2 cm diameter. It has been found that optimum sensitivity of rats in the body weight range 100-130 gms is obtained using a serum diluted with eight parts of physiological saline solution. This diluted solution is called antibody serum A.

The antigen to react with the antibody in serum A was prepared by removing N. brasiliensis worms from the gut of the infested rats, centrifuging the homogenate and collecting the supernatent liquor. This liquor was diluted with saline to give a protein content of 1 mg/ml and is known as solution B.

Charles River France/Fisons bred rats in the body weight range 100 to 130 gms were sensitised by intradermal injection of 0.1 mls of serum A into the right flank. Sensitivity was allowed to develop for 24 hours and the rats were then injected intravenously with 1 ml/100 gms body weight of a mixture of solution B (0.25 mls), Evans Blue dye solution (0.25 mls) and the solution of the compound under test (0.5 mls varying percentages of active matter). For each percentage level of active matter in the solution under test five rats were injected. Five rats were used as controls in each test. The dosages of the compound under test were selected so as to give a range of inhibition values.

Thirty minutes after injection of solution B the rats are killed and the skins removed and reversed. The intensity of the anaphylactic

reaction was assessed by comparing the size of the characteristic blue weal produced by spread of the Evans Blue dye from the sensitisation site, with the size of the weal in the control animals. The size of the weal was rated as 0 (no weal detected, i.e. 100% inhibition) to 4 (no difference in size of weal, i.e. no inhibition) and the percentage inhibition for each dose level calculated as:-

$$\% \text{ inhibition} = \frac{(\text{Control group score} - \text{treated group score}) \times 100}{\text{Control group score}}$$

The percentage inhibitions for the various dose levels were plotted graphically for each compound. From these graphs the dosage required to achieve a 50% inhibition of the anaphylactic reaction (ID_{50}) was determined. The results are shown in the following Table.

Table

Compound	ID_{50} mg/kg
Compound of Serial No 946,492	
Disodium 4,6-dioxo-10-propyl-4H,6H-pyrano-[3,2-g]quinoline-2,8-dicarboxylate	0.02
Compound of USP 3,959,289 (Ex 3)	
Disodium 1,4,6,9-tetrahydro-10-methyl-4,6-dioxypyrido[3,2-g]quinoline-2,8-dicarboxylate	0.056

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that wilful false statements and the like

so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardise the validity of the application or any patent issuing thereon.

this 9th day of April 1979

Declarant _____

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